

Amendment to the Specification

Please amend the specification as follows.

On page 1, after the title, please insert the following paragraph.

This application is a continuation of U.S. Application Serial No. 09/228,420, filed January 12, 1999, which is a divisional of U.S. Application Serial No. 08/465,971, filed June 6, 1995, now U.S. Patent No. 5,942,414.

On page 2, please replace the second full paragraph with the following replacement paragraph.

The membrane protein gene superfamily of G-protein coupled receptors (GPRs) has been characterized as having seven putative transmembrane domains. The domains are believed to represent transmembrane α -helices connected by extracellular or cytoplasmic loops. G-protein coupled receptors include a wide range of biologically active receptors, such as hormone, viral, growth factor and neuroreceptors.

On page 6, please insert before the first line of the page the following heading.

Brief Description of the Drawings

On page 6, please replace the second, third, and fourth paragraphs with the following replacement paragraphs.

~~Figure 1 shows~~ Figures 1A-E show the cDNA sequence (SEQ ID NO:1) and the corresponding deduced amino acid sequence (SEQ ID NO:2) of the G-protein coupled receptor of the present invention. The standard one-letter abbreviation for amino acids is used. Sequencing was performed using a 373 Automated DNA sequencer (Applied Biosystems, Inc.).

~~Figure 2 illustrates~~ Figures 2A-B illustrate an amino acid alignment of the G-protein coupled receptor of the present invention (top line of each comparative row, SEQ ID NO:2) and the human adrenergic α_2 A receptor (bottom line of each comparative row, SEQ ID NO:4).

In accordance with an aspect of the present invention, there is provided an isolated nucleic acid (polynucleotide) which encodes ~~for~~ the mature polypeptide having the deduced amino acid sequence of ~~Figure 1~~ Figures 1A-E (SEQ ID NO:2) or ~~for~~ the mature

polypeptide encoded by the cDNA of the clone (HIBEF51) deposited as ATCC Deposit No. 97182 with the ATCC[®], 10801 University Boulevard, Manassas, Virginia 20110-2209 on June 1, 1995. Since the strain referred to is being maintained under the terms of the Budapest Treaty, it will be made available to a patent office signatory to the Budapest Treaty.

On page 6, please replace the last paragraph, spanning pages 6 and 7, with the following replacement paragraph.

The polynucleotide of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encodes the mature polypeptide may be identical to the coding sequence shown in ~~Figure 1~~ Figures 1A-E (SEQ ID NO:1) or that of the deposited clone or may be a different cloning sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same mature polypeptide as the DNA of ~~Figure 1~~ Figures 1A-E (SEQ ID NO:1) or the deposited cDNA.

On page 7, please replace the first full paragraph with the following replacement paragraph.

The polynucleotide which encodes for the mature polypeptide of ~~Figure 1~~ Figures 1A-E (SEQ ID NO:2) or for the mature polypeptide encoded by the deposited cDNA may include: only the coding sequence for the mature polypeptide; the coding sequence for the mature polypeptide and additional coding sequence such as a leader or secretory sequence or a proprotein sequence; the coding sequence for the mature polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature polypeptide.

On page 7, please replace the third paragraph with the following replacement paragraph.

The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the polypeptide

having the deduced amino acid sequence of ~~Figure 1~~ Figures 1A-E (SEQ ID NO:2) or the polypeptide encoded by the cDNA of the deposited clone. The variant of the polynucleotide may be a naturally occurring allelic variant of the polynucleotide or a non-naturally occurring variant of the polynucleotide.

On page 7, please replace the last paragraph, spanning pages 7 and 8, with the following replacement paragraph.

Thus, the present invention includes polynucleotides encoding the same mature polypeptide as shown in ~~Figure 1~~ Figures 1A-E (SEQ ID NO:2) or the same mature polypeptide encoded by the cDNA of the deposited clone as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the polypeptide of ~~Figure 1~~ Figures 1A-E (SEQ ID NO:2) or the polypeptide encoded by the cDNA of the deposited clone. Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

On page 8, please replace the first full paragraph with the following replacement paragraph.

As hereinabove indicated, the polynucleotide may have a coding sequence which is a naturally occurring allelic variant of the coding sequence shown in ~~Figure 1~~ Figures 1A-E (SEQ ID NO:1) or of the coding sequence of the deposited clone. As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide.

On page 9, please replace the first full paragraph with the following replacement paragraph.

The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 70%, preferably at least 90%, and more preferably at least 95% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97%

identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode polypeptides which either retain substantially the same biological function or activity as the mature polypeptide encoded by the cDNA of ~~Figure 1~~ Figures 1A-E (SEQ ID NO:1) or the deposited cDNA(s).

On page 10, please replace the third paragraph and the last paragraph, spanning pages 10 and 11, with the following replacement paragraphs.

The present invention further relates to a receptor polypeptide which has the deduced amino acid sequence of ~~Figure 1~~ Figures 1A-E (SEQ ID NO:2) or which has the amino acid sequence encoded by the deposited cDNA, as well as fragments, analogs and derivatives of such polypeptide.

The terms “fragment,” “derivative” and “analog” when referring to the polypeptide of ~~Figure 1~~ Figures 1A-E (SEQ ID NO:2) or that encoded by the deposited cDNA, mean a polypeptide which either retains substantially the same biological function or activity as such polypeptide, i.e., functions as a receptor, or retains the ability to bind the ligand or the receptor even though the polypeptide does not function as an receptor, for example, a soluble form of the receptor. An analog includes an extracellular portion which can be cleaved from the transmembrane domain and intracellular portion to produce a soluble active peptide.

On page 11, please replace the second paragraph with the following replacement paragraph.

The fragment, derivative or analog of the polypeptide of ~~Figure 1~~ Figures 1A-E (SEQ ID NO:2) or that encoded by the deposited cDNA may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide which is employed for

purification of the mature polypeptide, or (v) one in which a fragment of the polypeptide soluble, i.e. not membrane bound, yet still binds ligands to the membrane bound receptor. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

On page 35, please replace the first paragraph under the heading “Example 1 Bacterial Expression and Purification of G-protein Coupled Receptor” with the following replacement paragraph.

The DNA sequence encoding the receptor, ~~ATCC~~ # ATCC[®] NO. 97182 is initially amplified using PCR oligonucleotide primers corresponding to the 5' and sequences of the processed receptor protein (minus the signal peptide sequence) and the vector sequences 3' to the receptor gene. Additional nucleotides corresponding to receptor were added to the 5' and 3' sequences respectively. The 5' oligonucleotide primer has the sequence 5'-CGGAATTCCTCCATGAACTCCACCTTGGAT-3' (SEQ ID NO:5) contains a an Eco RI restriction enzyme site followed by 18 nucleotides of receptor coding sequence starting from the presumed terminal amino acid of the processed protein codon. The 3' sequence 5'-CGGAAGCTTCGTCAGATATGACATCCATT-3' (SEQ ID NO:6) contains complementary sequences to HindIII site and is followed by 18 nucleotides of receptor. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc. 9259 Eton Avenue, Chatsworth, CA, 91311). PQE-9 encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. PQE-9 was then digested with Eco RI and HindIII. The amplified sequences were ligated into pQE-9 and inserted in frame with the sequence encoding for the histidine tag and the RBS. The ligation mixture was then used to transform E. coli strain available from Qiagen under the trademark M15/rep 4 by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press (1989). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies were selected. Plasmid DNA was isolated and confirmed by restriction analysis.

On page 37, please replace the second paragraph with the following replacement paragraph.

The DNA sequence encoding for the receptor, ATCC # ATCC[®] NO. 97182, was constructed by PCR on the original EST cloned using two primers: the 5' primer (5'-GTCCAAGCTTGCCACCATGAACTCCACCTTGGAT-3') (SEQ ID NO:5 7) contains a HindIII site followed by 18 nucleotides of receptor coding sequence starting from the initiation codon; the 3' sequence 5'-CTAGCTCGAGTCAAGCGTACTCTGGGACGTCGTATGGGTAGCAGATATGACATCCATTAAG-3' (SEQ ID NO:6 8) contains complementary sequences to Xho I site, translation stop codon, HA tag and the last 18 nucleotides of the receptor coding sequence (not including the stop codon). Therefore, the PCR product contains a HindIII site, receptor coding sequence followed by HA tag fused in frame, a translation termination stop codon next to the HA tag, and an Xho I site. The PCR amplified DNA fragment and the vector, pcDNAI/Amp, were digested with HindIII and Xho I restriction enzyme and ligated. The ligation mixture was transformed into E. coli strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037) the transformed culture was plated on ampicillin media plates and resistant colonies were selected. Plasmid DNA was isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant receptor, COS cells were transfected with the expression vector by DEAE-DEXTRAN method. (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the receptor HA protein was detected by radiolabelling and immunoprecipitation method. (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells were labelled for 8 hours with ³⁵S-cysteine two days post transfection. Culture media were then collected and cells were lysed with detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5). (Wilson, I. et al., Id. 37:767 (1984)). Both cell lysate and culture media were precipitated with a HA specific monoclonal antibody. Proteins precipitated were analyzed on 15% SDS-PAGE gels.

On page 38, please replace the first and second full paragraphs with the following replacement paragraphs.

The DNA sequence encoding the full length receptor protein, ~~ATCC~~ # ATCC[®] NO. 97182, was amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene:

The 5' primer has the sequence 5'-
CGGGATCCCTCCATGAACTCCACCTTCCAT (SEQ ID NO:7 9) and contains a Bam
HI restriction enzyme site (~~in bold~~) followed by 4 nucleotides resembling an efficient
signal for the initiation of translation in eukaryotic cells (J. Mol. Biol. 1987, 196, 947-950,
Kozak, M.), and just behind the first 18 nucleotides of the receptor gene (the initiation
codon for translation "ATG" is underlined).

On page 39, please replace the first paragraph with the following replacement paragraph.

The 3' primer has the sequence 5'-
CGGGATCCCGCTCAGATATGAGATCCATT-3' (SEQ ID NO:8 10) and contains the
cleavage site for the restriction endonuclease Bam HI and 18 nucleotides complementary
to the 3' non-translated sequence of the receptor gene. The amplified sequences were
isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101
Inc., La Jolla, Ca.). The fragment was then digested with the endonuclease Bam HI and
then purified as described in Example 1. This fragment is designated F2.

On page 57, please replace the Abstract with the following replacement abstract.

Human G-protein Coupled receptor HIBEF51 polypeptides and DNA (RNA)
encoding such polypeptides and a procedure for producing such polypeptides by
recombinant techniques is disclosed. Also disclosed are methods for utilizing such
polypeptides for identifying antagonists and agonists to such polypeptides and methods of
using the agonists and antagonists therapeutically to treat conditions related to the
underexpression and overexpression of the receptor polypeptides, respectively. Also
disclosed are diagnostic methods for detecting a mutation in the human G-protein Coupled
receptor HIBEF51 nucleic acid sequences and detecting a level of the soluble form of the
receptors in a sample derived from a host.